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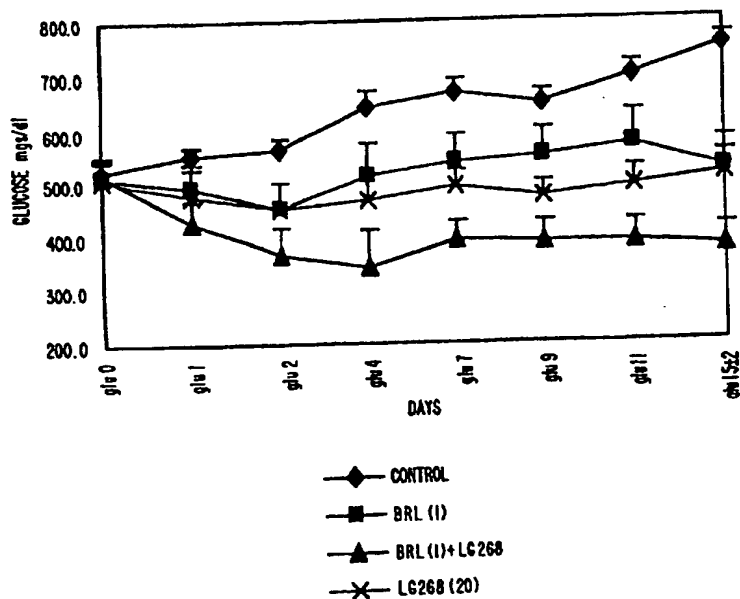
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(54) Title: TREATING NIDDM WITH RXR AGONISTS



(57) Abstract

This invention relates to methods and compositions for the treatment of non-insulin-dependent diabetes mellitus using an RXR agonist alone or in combination with a PPAR γ agonist such as a thiazolidinedione compound.

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TREATING NIDDM WITH RXR AGONISTS

FIELD OF THE INVENTION

This invention relates to methods and pharmaceutical
5 compounds for treating diabetes and related symptoms.

BACKGROUND OF THE INVENTION

Non-insulin-dependent diabetes mellitus (NIDDM, type
II diabetes) is characterized by abnormalities in insulin
10 secretion and insulin action. NIDDM constitutes 90-95% of the
approximately 6 million diagnosed diabetics in the United
States. NIDDM is characterized by hyperglycemia, the result
of insulin resistance in peripheral tissues (skeletal muscle
and adipose tissue), where insulin-stimulated
15 uptake/utilization of glucose is blunted, and in liver, where
insulin suppression of glucose output is insufficient. These
impairments in insulin action play an important role in the
development of elevated fasting blood glucose and glucose
intolerance.

20 Diet and exercise are first-line therapy for NIDDM
patients. NIDDM patients also take oral hypoglycemic drugs to
control blood glucose levels. The most widely used
hypoglycemic agents are various formulations of insulin and
sulfonylureas. A major drawback with these therapies is the
25 occurrence of potentially life-threatening hypoglycemia due to
hyperinsulinemia.

The hyperinsulinemia that can occur with these
therapies is also associated with an elevated risk of
cardiovascular disease, a major killer of diabetics.

Therefore, a need exists for antidiabetic drugs which do not increase circulating insulin concentrations.

A new class of compounds, thiazolidinediones, have been documented to effect antihyperglycemic activity by increasing insulin action rather than by promoting insulin secretion. Thiazolidinediones ameliorate insulin resistance and normalize plasma glucose and insulin (where elevated) without causing a hypoglycemic state, even at very high doses. The thiazolidinedione insulin sensitizers, e.g., ciglitazone, englitazone, pioglitazone, BRL 49653 (5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]methyl]-2,4-thiazolidinedione), and troglitazone, enhance insulin-mediated suppression of hepatic glucose output and insulin-stimulated glucose uptake and utilization by adipose tissue. Thiazolidinediones also change glucose transporter (e.g. Glut 4) expression to contribute to increased insulin responsiveness.

SUMMARY OF THE INVENTION

Applicant has found that RXR agonists mimic or enhance the antidiabetic effects of thiazolidinedione compounds. RXR agonists activate the transcriptional activity of RXR/PPAR γ heterodimers, increase insulin stimulated glucose uptake, lower the level of triglyceride, suppress the level of insulin, and increase the level of HDL cholesterol. Two RXR agonists have been shown to lower glucose, triglycerides and insulin levels in two established animal models of NIDDM, i.e. the *ob/ob* and *db/db* mice. Therefore, RXR agonists may be used as insulin sensitizers or insulin mimetics in the treatment of NIDDM and related symptoms.

In addition, the combination of an RXR agonist and a PPAR γ agonist, such as a thiazolidinedione, achieves synergistic activation of the RXR/PPAR γ heterodimers so as to enhance adipogenic and antidiabetic effects of PPAR γ . In db/db mice, the combination of an RXR agonist and a PPAR γ agonist was shown to lower the level of glucose more than individual compounds did.

Therefore, the present invention relates to methods and compositions for treating a host having NIDDM or insulin resistant diabetes by administering to the host a composition containing a pharmaceutically effective amount of an activator of the RXR/PPAR γ heterodimer, including, but not limited to, an RXR agonist. The host may be a human patient or an animal model of human NIDDM. The compositions of this invention are adapted to cure, improve or prevent one or more symptoms of NIDDM in the host. A preferred drug is highly potent and selective with low toxicity. In this regard, those skilled in the art will recognize NIDDM as an example of a metabolic disease that can be treated with the RXR agonist-containing compounds and compositions of the present invention. Other examples of metabolic diseases treatable with the compounds and compositions of the present invention include, but are not limited to, obesity and thyroid hormone abnormalities.

By "pharmaceutically effective amount" is meant an amount of a pharmaceutical compound or composition having a therapeutically relevant effect on NIDDM. A therapeutically relevant effect relieves to some extent one or more symptoms of NIDDM in a patient or returns to normal either partially or completely one or more physiological or biochemical parameters

associated with or causative of NIDDM, e.g. increasing the sensitivity of cellular response to circulating insulin, curing, reducing, or preventing one or more clinical symptoms of NIDDM, including, but not limited to, hyperglycemia, hyperinsulinemia and hypertriglyceridemia. In a preferred embodiment, a pharmaceutically effective amount of a compound or composition means an amount that increases the uptake of glucose by adipose tissue or muscle tissue. In another preferred embodiment, a pharmaceutically effective amount of a compound or composition means an amount that increases the uptake of triglyceride by adipose tissue.

By "activator of the RXR/PPAR γ heterodimer" is meant a compound or composition which when combined with the RXR/PPAR γ heterodimer increases the transcriptional regulation activity of the heterodimer, as measured by an assay known to one skilled in the art, including, but not limited to, the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355, WO91/06677, WO92/05447, WO93/11235, WO95/18380, PCT/US93/04399, PCT/US94/03795 and CA 2,034,220, which are incorporated by reference herein. It includes, but is not limited to, compounds that bind RXR, PPAR γ , or both.

By "RXR agonist" is meant a compound or composition which when combined with RXR homodimers or heterodimers increases the transcriptional regulation activity of RXR, as measured by an assay known to one skilled in the art, including, but not limited to, the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355,

WO91/06677, WO92/05447, WO93/11235, WO95/18380,
PCT/US93/04399, PCT/US94/03795 and CA 2,034,220, which are
incorporated by reference herein. It includes, but is not
limited to, compounds that preferentially activate RXR over
5 RAR (i.e. RXR specific agonists), and compounds that activate
both RXR and RAR (i.e. pan agonists). It also includes
compounds that activate RXR in a certain cellular context but
not others (i.e. partial agonists). Compounds disclosed or
described in the following articles, patents and patent
10 applications which have RXR agonist activity are incorporated
by reference herein: U.S. patents 5,399,586 and 5,466,861,
WO96/05165, PCT/US95/16842, PCT/US95/16695, PCT/US93/10094,
WO94/15901, PCT/US92/11214, WO93/11755, PCT/US93/10166,
PCT/US93/10204, WO94/15902, PCT/US93/03944, WO93/21146,
15 provisional applications 60,004,897 and 60,009,884, Boehm, et
al. J. Med. Chem. 38(16):3146-3155, 1994, Boehm, et al. J.
Med. Chem. 37(18):2930-2941, 1994, Antras et al., J. Biol.
Chem. 266:1157-1161 (1991), Salazar-Olivo et al., Biochem.
Biophys. Res. Commun. 204:157-263 (1994) and Safanova, Mol.
20 Cell. Endocrin. 104:201-211 (1994). RXR specific agonists
include, but are not limited to, LG 100268 (i.e. 2-[1-
(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-
cyclopropyl]-pyridine-5-carboxylic acid) and LGD 1069 (i.e. 4-
[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-2-
25 carbonyl]-benzoic acid), and analogs, derivatives and
pharmaceutically acceptable salts thereof. The structures and
syntheses of LG 100268 and LGD 1069 are disclosed in Boehm, et
al. J. Med. Chem. 38(16):3146-3155, 1994, incorporated by
reference herein. Pan agonists include, but are not limited

to, ALRT 1057 (i.e. 9-cis retinoic acid), and analogs, derivatives and pharmaceutically acceptable salts thereof.

In a preferred embodiment, the pharmaceutical composition also contains a pharmaceutically effective amount of a PPAR γ agonist. Alternatively, a second composition containing a pharmaceutically effective amount of a PPAR γ agonist is administered to the host separately. In a further preferred embodiment, a compound having agonist activity for both RXR and PPAR γ is used.

By "PPAR γ agonist" is meant a compound or composition which when combined with PPAR γ increases a reaction typical for the receptor, e.g., transcriptional regulation activity, as measured by an assay known to one skilled in the art, including, but not limited to, the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784 and 5,071,773 and Lehmann, et al., J. Biol. Chem. 270:12953-12956 (1995), which are incorporated by reference herein. A preferred PPAR γ agonist is a thiazolidinedione compound, including, but not limited to, BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075, darglitazone, and analogs, derivatives and pharmaceutically acceptable salts thereof. Compounds disclosed in Tontonez et al., Genes & Develop. 8:1224-1234 (1994), Tontonez et al., Cell 79:1147-1156 (1994), Lehmann et al., J. Biol. Chem. 270(22):1-4, 1995, Amri et al., J. Lipid Res. 32:1449-1456 (1991), Amri et al., J. Lipid Res. 32:1457-1463, (1991) and Grimaldi et al., Proc. Natl. Acad. Sci. USA 89:10930-10934 (1992) are incorporated by reference herein.

In a further preferred embodiment, the pharmaceutical composition also contains a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer, or insulin mimetic.

5 Alternatively, a composition containing a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer, or insulin mimetic is administered to the host separately.

10 A composition containing a pharmaceutically effective amount of an active ingredient may be administered orally or systemically to a host. In a preferred embodiment, it is administered orally.

In another aspect, this invention features a pharmaceutical composition for treating NIDDM containing a
15 pharmaceutically effective amount of an RXR agonist; and a pharmaceutically acceptable carrier adapted for a host having NIDDM. In a preferred embodiment, the pharmaceutical composition also includes a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin
20 sensitizier, insulin mimetic or PPAR γ agonist.

In a preferred embodiment, the composition is held within a container which includes a label stating to the effect that the composition is approved by the FDA in the United States (or an equivalent regulatory agency in a foreign
25 country) for treating NIDDM or for treating hyperglycemia, hyperinsulinemia or hypertriglyceridemia. Such a container provides a therapeutically effective amount of the active ingredient to be administered to a host.

In another aspect, this invention features methods for screening for candidate compounds useful for treating NIDDM. These methods select compounds or compositions which when combined with the RXR/PPAR γ heterodimer increase the transcriptional regulation activity of the heterodimer, as measured by an assay known to one skilled in the art, including, but not limited to, the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355, WO91/06677, WO92/05447, WO93/11235, WO95/18380, PCT/US93/04399, PCT/US94/03795 and CA 2,034,220, which are incorporated by reference herein. In one example, a candidate compound such as a potential RXR agonist is administered to an adipocyte or a preadipocyte. The level of lipid in the cell is measured, and an increased accumulation of lipid after the treatment with the candidate compound indicates that the candidate compound is useful for treating NIDDM. In preferred embodiments, the level of lipid is measured by oil red O staining or detecting the level of triglyceride in the cell.

In another example, a candidate compound such as a potential RXR agonist is administered to an adipocyte or a preadipocyte and the transcription level of a adipocyte specific gene (e.g. lipoprotein lipase gene or PPAR γ gene) is measured. An increased transcription of the adipocyte specific gene after the treatment with the candidate compound indicates that the candidate compound is useful for treating NIDDM.

In yet another example, a candidate compound such as a potential RXR agonist is administered to an adipocyte or a preadipocyte and the level of glucose uptake is measured. An increased glucose uptake after the treatment with the candidate compound indicates that the candidate compound is useful for treating NIDDM. Alternatively, both the candidate compound and insulin are administered to the cell and the level of glucose uptake is compared to that in the same cell treated with insulin alone. A higher level of glucose uptake in the cell treated by the candidate compound and insulin indicates that the candidate compound is an insulin sensitizer and useful for treating NIDDM.

Other features and advantages of the invention will be apparent from the following detailed description of the invention, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a is a graph showing the extent of adipocyte differentiation in 3T3-LI cells as measured by the levels of triglyceride in 3T3-LI preadipocytes treated with various combinations of an RXR agonist (LG 100268), a PPAR γ agonist (thiazolidinedione compound BRL 49653), and insulin. The retinoid and BRL 49653 were used at 1 μ M.

Figure 1b is a graph showing the extent of adipocyte differentiation in 3T3-LI cells as measured by the level of triglyceride in 3T3-LI preadipocytes treated with various combinations of an RXR agonist (LGD 1069), a PPAR γ agonist (thiazolidinedione compound BRL 49653), and insulin. The retinoid and BRL 49653 were used at 1 μ M.

Figure 2a is a graph showing the level of LPL mRNA in 3T3-LI cells treated with various combinations of an RXR agonist (LG 100268), a PPAR γ agonist (thiazolidinedione compound BRL 49653), and insulin. The retinoid and BRL 49653 were used at 1 μ M.

Figure 2b is a graph showing the level of PPAR γ mRNA in 3T3-LI cells treated with various combinations of an RXR agonist (LG 100268), a PPAR γ agonist (thiazolidinedione compound BRL 49653), and insulin. The retinoid and BRL 49653 were used at 1 μ M.

Figure 3 is a graph showing the level of glucose in *db/db* mice treated with LG 100268, BRL 49653, and a combination of LG 100268 and BRL 49653, respectively.

Figure 4 is a graph showing the level of triglycerides in *db/db* mice treated with LG 100268, BRL 49653, and a combination of LG 100268 and BRL 49653, respectively.

Figure 5 is a graph showing the level of HDL cholesterol in *db/db* mice treated with LG 100268, BRL 49653, and a combination of LG 100268 and BRL 49653, respectively.

Figure 6 is a graph showing the level of triglycerides in *ob/ob* mice treated with LGD 1069, LG 100268, and BRL 49653, respectively.

Figure 7 is a graph showing the level of glucose in *ob/ob* mice treated with LGD 1069, LG 100268, and BRL 49653, respectively.

Figure 8 is a graph showing the level of insulin in *ob/ob* mice treated with LGD 1069, LG 100268, and BRL 49653, respectively.

DETAILED DESCRIPTION OF THE INVENTION

TZD achieves antidiabetic and adipogenic effects through PPAR γ

Thiazolidinediones are insulin sensitizers that significantly reduce glucose and lipid levels in animal models of NIDDM and obesity (Kees, et al., J. Medicinal Chem. 38(4):617-628, 1995; Willson, et al., J. Medicinal Chem. 39(3):665-668, 1996; Young, et al. Diabetes 44:1087-1092, 1995). Thiazolidinediones improve glucose utilization without stimulating insulin release.

For example, repeated administration of BRL 49653 to obese mice improves glycemic control by increasing insulin responsiveness of target tissues. BRL 49653 potentiates insulin-stimulated glucose transport in adipocytes from insulin-resistant obese mice, both by increasing insulin receptor number and by facilitating translocation of GLUT4, from an expanded intracellular pool, to the cell surface.

Thiazolidinediones are also selective PPAR γ agonists (Lehmann, et al. J. Biol. Chem. 270(22):1-4, 1995). Comparison of the EC₅₀ for activation of PPAR γ with the minimum effective dose (MED) for anti-hyperglycemic activity revealed a significant correlation. The correlation between in vitro PPAR γ activity and in vivo antihyperglycemic activity of thiazolidinediones implicates PPAR γ as the molecular target for the antidiabetic effects of thiazolidinediones.

PPAR γ is a member of the nuclear receptor superfamily of ligand-activated transcription factors. It is expressed in an adipose-specific manner and its expression is induced early during the differentiation of several

preadipocyte cell lines. Forced expression of PPAR γ in fibroblasts resulted in adipocyte differentiation.

In addition to insulin sensitizing activity, thiazolidinediones have marked adipogenic effects on
5 preadipocyte and mesenchymal stem cells (Tontonoz, et al., Cell 79:1147-1156, 1994). Treatment of C3H/10T1/2 cells with BRL 49653 resulted in efficient adipocyte differentiation, showing that ligand-mediated activation of PPAR γ is sufficient to initiate the adipogenic signaling cascade in a mesenchymal
10 stem cell line. PPAR γ is the molecular target for the adipogenic effects of thiazolidinediones.

Adipogenesis plays a role in the development of NIDDM, which is characterized by not only unbalanced glucose homeostasis, but also elevated levels of circulating lipids.
15 Increases in lipid levels have been shown to interfere with glucose disposal.

Adipocytes are highly specialized cells that play a critical role in lipid metabolism and energy homeostasis. Their primary role is to store triglycerides in times of
20 caloric excess and to mobilize this reserve during periods of nutritional deprivation.

Adipocyte differentiation is characterized by a coordinate increase in adipocyte-specific gene expression. PPAR γ is specifically expressed in adipocytes. Its expression
25 is induced early during the course of differentiation of several preadipocyte cell lines. Forced expression of PPAR γ in fibroblasts resulted in adipocyte differentiation.

Synergistic effects of RXR and PPAR γ on adipogenesis

Expression of PPAR γ is induced early during the differentiation of cultured adipocyte cell lines and is expressed at very high levels specifically in adipose tissue. PPAR γ regulates adipogenesis by modulating the transcription of other adipocyte-specific genes, e.g. adipocyte P2 gene (aP2 gene). aP2 gene encodes an intracellular lipid-binding protein and is expressed exclusively in adipose cells.

A 518-bp DNA fragment from the 5'-flanking region of the aP2 gene has been identified as an enhancer that directs high-level adipocyte-specific gene expression in both cultured cells and transgenic mice. A pair of elements in the aP2 enhancer, ARE6 and ARE7, bind a nuclear factor termed ARF6 that is detected only in nuclear extracts derived from adipocytes. The ARF6-binding sites are both necessary and sufficient for adipocyte-specific expression, suggesting that the trans-acting factor ARF6 functions as a differentiation-dependent and tissue-specific switch for the aP2 enhancer (Tontonoz, et al. Genes & Development, 8:1224-1234, 1994).

The ARF6 recognition sequence resembles a type of nuclear hormone receptor-binding site known as DR-1 (direct repeat with 1-nucleotide spacer). This motif has been shown to preferentially bind heterodimers of RXR and COUP-TF and heterodimers of RXR and the PPARs. DNA mobility retardation experiments using various HRE sequences as competitor demonstrated that ARF6 preferentially recognizes DR-1 sites.

ARF6 has been identified as a heterodimeric complex of RXR α and PPAR γ . It has been shown that PPAR γ and RXR α form heterodimers on ARF6-binding sites in vitro. Forced

expression of these factors in transient transfections is sufficient to activate the adipocyte-specific aP2 enhancer in nonadipose cells such as fibroblasts. This activation is potentiated by peroxisome proliferators, fatty acids, and 9-cis retinoic acid. Antiserum to RXR α specifically inhibits ARF6 activity in adipocyte nuclear extracts.

Cotransfection of the RXR α expression vector and the PPAR γ expression vector has a synergistic effect to activate the aP2 enhancer in nonadipose cells. Maximal activation of the aP2 enhancer is observed when both PPAR γ , RXR α and their agonists are present.

Without being bound by any theory, applicant proposes that an RXR agonist affects glucose usage in tissues through the synergistic effects of RXR and PPAR γ heterodimers. The RXR/PPAR γ heterodimers, when activated by an RXR agonist or a combination of an RXR agonist and a PPAR γ agonist, induce adipogenesis and modulates the levels of glucose and triglyceride uptake. Alternatively or in addition, the RXR/PPAR γ heterodimers, when activated by an RXR agonist or a combination of an RXR agonist and a PPAR γ agonist, regulate signaling molecules secreted by adipose tissue such as tumor necrosis factor- α or leptin, which in turn modulates glucose metabolism in other tissues.

Using RXR agonists to mimic or enhance the antidiabetic effects of thiazolidinediones

PPAR α , β and γ all form heterodimers with RXRs. These RXR/PPAR heterodimers bind to DNA and regulate transcription activity. RXR activators cooperate with PPAR α

activators to activate the activity of PPAR α protein (Kliwer, et al. Nature 358:771-774 (1992) and Mukherjee, et al. Steroid Biochem. Molec. Biol. 51:157-166 (1994)).

5 In this invention, a similar synergistic activation was observed with a PPAR γ activator and several RXR activators.

According to this invention, RXR agonists, e.g. LGD 1069, ALRT 1057 and LG 100268, may be utilized in the treatment of diabetes. We examined four independent
10 parameters for the effects of RXR agonists, i.e. morphological changes, lipid accumulation, regulation of gene expression and increased glucose uptake.

Two pre-adipocyte cell lines were used to test the theory of RXR activation in the PPAR γ /RXR heterodimer. 3T3-LI
15 and C3H/10T1/2 cells were obtained from ATCC and are derived from mouse embryo. They are contact inhibited and can be induced to differentiate into adipocyte cells containing large lipid droplets within the cytoplasm. Adipocyte differentiation can be observed by oil red O staining which
20 stains the lipid droplets within the cytoplasm red. The extent of adipocyte differentiation can then be monitored by microscope observation.

For a more quantitative assay and rapid screening of compounds, a 96-well plate assay was developed to quantitate
25 the amount of triglyceride produced by the differentiating adipocytes. In this assay, cells are grown as a monolayer to confluence on a 96-well plate and treated with BRL 49653, insulin, and retinoids alone or in various combinations. These treatments induce differentiation to different extent in

both 3T3-LI and C3H/10T1/2 cells. The level of triglyceride accumulation can then be measured via an enzymatic color reaction which can be read in a plate reader.

5 A third measure of adipocyte differentiation is to examine regulation of gene expression. The mRNA expression levels of both PPAR γ and lipoprotein lipase (LPL) have been shown to be modulated during adipocyte differentiation. Northern blot analysis was used to dissect the molecular aspects of how retinoids effect target genes of
10 differentiating adipocytes. PPAR γ , lipoprotein lipase (LPL), and β -actin (loading control) mRNA levels were monitored after cells were treated with thiazolidinediones and retinoids.

A fourth indicator for the utility of a compound in treating NIDDM or insulin-resistant diabetes is the compound's
15 ability to enhance insulin-stimulated glucose uptake. Labeled 2-deoxyglucose (2-DOG, a glucose analog) assay was performed with a preadipocyte cell line in the presence of insulin and a candidate compound to measure the level of 2-DOG incorporation.

20

A) Retinoid Modulation of Lipid Accumulation in 3T3-LI Cells Stained with Oil Red O

Table 1 shows the percent of 3T3-LI cells that had differentiated into adipocytes as observed by oil red O
25 staining assay. BRL 49653 and LG 100268 were used at 1 μ M, insulin was used at 0.01 mg/ml. Wells treated with LG 100268 had bigger redder lipid droplets within the cytoplasm.

BRL 49653 and LG 100268 treatment alone induced 50% of the cells to differentiate into adipocytes. This was increased dramatically with the addition of insulin. Insulin in combination with BRL induced 80% of the 3T3-LI cells into adipocytes while the combination of insulin and LG 100268 induced 90% of the cells to differentiate. When BRL 49653 was used in combination with LG 100268, an RXR agonist, the amount of adipocytes differentiation was also increased dramatically. Other RXR agonists mimic the activity of LG 100268. For example, the addition of ALRT 1057 (pan agonist) or LGD 1069 (RXR specific agonist) in combination with BRL 49653 increased the amount of differentiation, albeit to a lesser extent than the strong RXR agonist LG 100268. The combination of insulin with BRL 49653 and LGD 1069 had a strong differentiating effect (95%) on the 3T3-LI cells.

B) Retinoid Modulation of Triglyceride Content in Differentiated Adipocytes

The retinoid modulation of lipid formation was quantitated by monitoring triglyceride formation. Figures 1a and 1b show triglyceride accumulation in 3T3-LI cells treated with a retinoid (LG 100268 or LGD 1069) alone or in combination with a thiazolidinedione and insulin. Retinoids and BRL 49653 were used at 1 μ M, insulin was used at 0.01 mg/ml for all experimental combinations.

Insulin, BRL 49653 and retinoids all induced some triglyceride accumulation when used alone, with LG 100268 giving the largest response. The addition of retinoids (LGD 1069, LG 100268) with the thiazolidinedione (BRL 49653) to the

assay increased the amount of triglyceride accumulation in 3T3-LI differentiating adipocytes. This was also observed when BRL 49653 or LG 100268 was used in combination with insulin. The largest accumulation of triglyceride was seen in the cells treated with LG 100268, BRL 49653 and insulin together. Similar results were observed when LGD 1069 replaced LG 100268 in the study. These results concur with those obtained in the oil red O staining assays.

10 C) PPAR γ and LPL mRNA modulation in differentiating 3T3-LI cells

Adipocyte specific genes were monitored via northern blot analysis. Figure 2a and 2b show the expression pattern of LPL (lipoprotein lipase) mRNA and PPAR γ mRNA in cells that were treated for 7 days with BRL 49653 (1 μ M), LG 100268 (1 μ M) and insulin (0.01 mg/ml), alone or in combination.

Northern blot analysis shows an increase in the relative signal normalized to β -actin of both LPL and PPAR γ mRNA expression in cells treated with either compound alone. There was a three to five fold increase in mRNA levels for these adipocyte target genes demonstrating that transcriptional regulation occurs with treatment by insulin, BRL 49653 and LG 100268. Combination of insulin, BRL 49653 and LG 100268 did not further enhance the mRNA level.

25 These data demonstrate that, in 3T3-LI cells, RXR agonists induce adipocyte differentiation by themselves or in combination with thiazolidinediones or insulin. RXR agonists enhance the activity of thiazolidinediones and insulin. Three independent measurements support that RXR agonists contribute

to the modulation of the RXR/PPAR γ heterodimer in regulating adipocyte differentiation and useful in treating NIDDM.

D) LG 100268 enhances insulin stimulated glucose uptake in 3T3-L1 cells

5 A murine preadipocyte cell line, 3T3-L1, is widely used to study glucose uptake, adipogenesis, and has been used in the characterization of thiazolidinediones and other PPAR γ activators. Insulin stimulated uptake of labeled 2-deoxyglucose (2-DOG, a glucose analog) was observed in 3T3-L1
10 cells treated with BRL 49653 or LG 100268.

3T3-L1 cells were treated with BRL 49653 (10 μ M) or LG 100268 (1 μ M) for 10 days. Insulin was added to the cells at a concentration of 0.01 mg/ml for 5 days, thereafter no
15 insulin was added. Labeled 2-deoxyglucose uptake assay was performed (Szalkowski, et al., J. Endocrin. 136:1474-1481, 1995). The level of incorporated labeled 2-DOG was normalized to the amount of total cellular protein. About 5-fold increase in 2-DOG uptake was observed in 3T3-L1 cells treated
20 with BRL 49653 alone. About 2-fold increase in 2-DOG uptake was observed in 3T3-L1 cells treated with LG 100268 alone.

This experiment shows that an RXR agonist increases insulin mediated glucose uptake in 3T3-L1 cells like a known insulin sensitizier, a thiazolidinedione. It demonstrates
25 directly that RXR agonists can be used to treat a major symptom of NIDDM, i.e. insulin resistance. Other RXR agonists useful for the treatment of NIDDM can be confirmed using this assay.

E) Retinoid Modulation of Lipid Accumulation in
C3H/10T1/2 Cells Stained with Oil Red O

To further assess the retinoid regulation of adipocyte function we examined C3H/10T1/2 cells, which are mouse embryo fibroblast/multipotent stem cells that can be induced to differentiate into adipocytes or muscle cells. Table 2 shows the percent of C3H/10T1/2 cells that have differentiated into adipocytes as observed by oil red O staining assay. Experiment A was conducted in the presence of BRL 49653. Experiment B was conducted in the presence of both BRL 49653 and insulin. ALRT 1057 and LGD 1069 were used at 1 μ M and TTNPB (RAR selective compound) was used at 10 nM concentration. Insulin was used at 0.01 mg/ml; and BRL 49653 was used at 0.1, 1 and 10 μ M. C3H/10T1/2 cells were treated for 7 days, cells were stained with oil red O and observed under the microscope.

Morphological changes were observed when the cells were treated with retinoids alone, even though the cells did not fully differentiate into adipocytes. BRL 49653 alone caused no more than 10 percent of the cells to undergo adipocyte differentiation. However, when BRL 49653 was used in combination with an RXR agonist, LGD 1069 or ALRT 1057, there was a large increase in the amount of differentiation, the same effect was seen when BRL 49653 was used in combination with insulin.

The RAR agonist, TTNPB (which does not activate RXR), did not have the same effect as RXR agonist LGD 1069 and ALRT 1057. In fact, TTNPB inhibited the differentiation induced by BRL 49653 or BRL/insulin.

The above experiments show that a thiazolidinedione (BRL 49653) alone induces a minimal amount of differentiation in C3H/10T1/2 cells. However, when BRL 49653 is used in combination with a retinoid such as LG 100268, LGD 1069, or ALRT 1057, which are RXR agonists, differentiation is dramatically increased. This is not seen with the pure RAR agonist, TTNPB. These data support that the PPAR γ /RXR heterodimers, which drive the adipocyte differentiation process, can be activated and enhanced by the binding of an RXR agonist. RXR agonists are useful for modulating the levels of glucose and triglyceride uptake.

Using RXR agonists to lower levels of glucose and triglycerides in animal models of NIDDM

(A) In vivo experiment with db/db mice

Animals: Strain Diabetic C57BLKS/J - m +/+db, 82 mice

Source: Jackson Lab

Stock number 000642

Genotype m +/+db xm +/+db

DOB 6/19/96 \pm 3d, DOA 7/23/96 - 34d old

Date of study: 8/5/96 - 8/21/96, 44-63d old

Mice were identified by an ear punch code (#1-82) and separated into 8 groups (A-H). Each group consisted of 10 mice separated into 4 cages with 2-3 mice/cage. Several mice were lost during the course of the study from fluid injections into the lungs resulting in 2 groups with 9 mice/group. Control group C consisted of 12 mice. The mice were fed pelleted Purina Lab chow #5015 containing 3.83kcal/g with a caloric composition of 56% carbohydrate, 26% fat and 18% protein. Food and water were provided ad libitum. Food

intake/cage was measured over selected periods and expressed as g food consumed/100g mouse/day.

On days of study, food was removed from the cages at selected intervals of time between 6:15 and 7:00 AM. Animal weights were recorded 2h after start of fast and blood samples taken after a 3h fast. Blood was drawn from a cut at the tip of the tail and collected into a heparinized capillary tube (approx. 75 μ l volume). After centrifugation, the hematocrit was read in a microcapillary reader, recorded and the tube broken for recovery of plasma for analysis of glucose, triglyceride and insulin concentration.

Blood samples were collected on days 0, 3, 7, 10, and on the final day of study, days 13-15. After collection of blood samples on day 0, animals were refed their Purina chow diet and subsequently gavaged with control solution in group C and one of seven test solutions in groups designated A, B, D-H. The volume administered was equivalent in each group averaging .6ml/42g mouse (.01429 ml/g). The various solutions were gavaged daily to their respective groups based on the animals weight taken that morning or the weight of the animal taken on the preceding day of weighing. To assess alterations in plasma FFA, on day 10 an addition 75 μ l blood sample was collected into an EDTA-coated capillary tube immediately after collecting the basal heparinized capillary tube sample.

On the final day of study, mice were not gavaged with test solution. The last gavage was administered on the day preceding the final day, i.e., day 12 for animals terminated on day 13, day 13 for animals terminated on day 14

and day 14 for animals terminated on day 15. Terminal blood was collected by decapitation to provide serum for assessment of HDL.

5 Results:

Figure 3 shows that BRL49653 and LG100268 each independently lowered the level of glucose in *db/db* mice. In addition, the combination of BRL49653 and LG100268 lowered the glucose level more than each compound did by itself. BRL
10 49653 (1 mg/kg) and LG 100268 (20 mg/kg) lowered the level of glucose by 40% by day 15 in comparison to control. The thiazolidinedione BRL 49653 at 1 mg/kg showed similar efficacy. The combination of an RXR activator and a PPAR
15 activator showed greater efficacy, leading to almost 50% drop in the level of glucose. The effect of the combination was rapid, glucose levels were reduced by day 1 of the study and reached a steady state by day 4. This indicates a rapid resetting of the steady state levels of glucose homeostasis. Therefore, RXR activators enhance the efficacy of PPAR
20 activators, and vice versa.

Figure 4 shows that RXR activators lowered the level of triglycerides in *db/db* mice. LG 100268 (20 mg/kg) lowered the level of triglycerides 40% by day 15 of the study. BRL
25 49653 (1 mg/kg) showed similar efficacy. The combination of these two compounds worked even better.

Figure 5 shows that RXR activators modulators increased the level of HDL cholesterol in *db/db* mice. LG 100268 (20 mg/kg) increased HDL-cholesterol levels (20%) in comparison to controls in *db/db* mice. BRL 49653 caused an

equivalent level of increase. The combination of these two compounds showed a higher increase. HDL-C was measured by the precipitation method using kits obtained from Bohringer-Mannheim (catalog # 543004 and 427578).

5

(B) In vivo experiment with ob/ob mice

Animals: Strain Obese C57 BL/6J-*Lep^{ob(4)}*, 121 mice

Source: Jackson Lab Stock number 000632

Genotype *Lep^{ob(4)}/+* x *Lep^{ob(4)}/+*

10

DOB 5/22/96±3d, DOA 7/2/96 - 41d old

Date of study: 7/21/96 - 8/2/96 - 49-70d old

Mice were identified by an ear punch code (#1-121) and separated into 12 groups. Each group consisting of 10 mice. (one mouse was terminated before start of the study because of bad teeth causing initial weight loss. As in preceding studies, mice in each group were housed in 4 cages (2-3 mice/cage) and provided water and Purina Lab chow #505\15 ad libitum.

15

20

25

On days of study, food was removed from the cages at selected intervals of time between 6:15 and 7:00 AM. Animal weights were recorded 2h after start of fast and blood samples taken after a 3h fast. Blood was drawn from a cut at the tip of the tail and collected into a heparinized capillary tube (approx. 75µl volume). After centrifugation, the hematocrit was read in a microcapillary reader, recorded and the tube broken for recovery of plasma for analysis of glucose, triglyceride and insulin concentration.

Blood samples were collected on days 0., 0, 3, 6, 8, 10, 14±1 and final collections were made on days 15, 16 or 17. FFA samples were collected on day 10 by drawing a second 75µl blood sample in a non-heparinized tube coated with EDTA. This tube was collected immediately after the heparinized tube collection.

Animals in groups H were administered control gavage solution (0.6 ml/42 g) daily commencing on day 0 and ending on the day preceding the final day. 11 test solutions were administered to animals in groups designated A-G, I-L. All gavage solutions were administered after refeeding on days in which mice were fasted for blood sampling.

Results:

Figure 6 shows that RXR modulators lowered the level of triglycerides in *ob/ob* mice. RXR activators LGD 1069 (30 mg/kg) and LG 100268 (20 mg/kg) lowered the level of triglycerides by 34% and 60% respectively in *ob/ob* mice by day 14 of the study. BRL 49653 was also able to lower the level of triglycerides, although not as efficacious as LG100268.

Figure 7 shows that RXR modulators lowered the level of glucose in *ob/ob* mice. RXR activators LGD 1069 at 30 mg/kg and LG 100268 at 20 mg/kg lowered the level of glucose nearly 50% in comparison to control. The level of glucose was reduced to almost euglycemic levels by day 14 of the study. BRL 49653 (0.4 mg/kg) showed similar efficacy.

Figure 8 shows that RXR modulators, LGD 1069 (30 mg/kg) and LG 100268 (20 mg/kg), lowered the level of insulin in *ob/ob* mice. LG100268 lowered the level of insulin by 66% by day 14 of the study. LGD 1069 showed lower efficacy.

5 There was a very rapid effect of the compounds since the level of insulin started to drop in day 1.

Pharmaceutical Formulations and Modes of Administration

The particular compound that affects the disorders
10 or conditions of interest can be administered to a patient either by themselves, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of a agent or agents such as
15 these is administered. A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient.

The compounds also can be prepared as
20 pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include acid addition salts such as those containing hydrochloride, sulfate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-
25 toluenesulfonate, cyclohexylsulfamate and quinate. (See e.g., PCT/US92/03736). Such salts can be derived using acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid,

benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid. These salts can be prepared by standard techniques. For example, the free base form of the compound is first dissolved in a suitable solvent such as an aqueous or aqueous-alcohol solution, containing the appropriate acid. The salt is then isolated by evaporating the solution. In another example, the salt is prepared by reacting the free base and acid in an organic solvent.

Carriers or excipients can be used to facilitate administration of the compound, for example, to increase the solubility of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents.

In addition, the molecules tested can be used to determine the structural features that enable them to act on the RXR/PPAR γ heterodimer, and thus to select molecules useful in this invention. Those skilled in the art will know how to design drugs from lead molecules, using techniques such as those disclosed in PCT publication WO 94/18959, incorporated by reference herein.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio

LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl et al., in The Pharmacological Basis of Therapeutics, 1975, Ch. 1 p. 1). It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific conditions being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the

invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or

solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone

(PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings.
5 For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be
10 added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft,
15 sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft
20 capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Liposomes may be used for encapsulated delivery.

Pharmaceutical formulations disclosed or described
25 in Boehm, et al., WO94/15902 are incorporated by reference herein.

All publications referenced are incorporated by reference herein, including the nucleic acid sequences and amino acid sequences listed in each publication. All the

compounds disclosed and referred to in the publications mentioned above are incorporated by reference herein, including those compounds disclosed and referred to in articles cited by the publications mentioned above.

5 Other embodiments of this invention are disclosed in the following claims.

TABLE 1: Oil Red O Staining in 3T3-L1 Differentiating
Adipocytes

	Treatment	Percent of Differentiated Cells
5 10	Control	0.1
	BRL 49653	50
	Insulin	20
	LG 100268	50
	BRL+Insulin	80
	BRL+LG 100268	90
	LG 100268+Insulin	90

TABLE 2: Retinoid Modulation of Adipocyte differentiation in
C3H/10T1/2 Cells

	Treatment	Experiment A (+BRL) % differentiated cells	Experiment B (+BRL+Insulin) % differentiated cells
5	BRL (10 μ M)	10	80
	BRL (1 μ M)	8	80
	BRL (0.1 μ M)	5	60
10	BRL (10 μ M)	10	80
	+ALRT 1057 (1 μ M)	80	99
	+LGD 1069 (1 μ M)	80	99
	+TTNPB (10nM)	5	50
15	BRL (1 μ M)	8	80
	+ALRT 1057 (1 μ M)	65	90
	+LGD 1069 (1 μ M)	65	90
	+TTNPB (10nM)	2	30
20	BRL (0.1 μ M)	5	60
	+ALRT 1057 (1 μ M)	45	85
	+LGD 1069 (1 μ M)	45	90
	+TTNPB (10nM)	0.2	

Undifferentiated controls 0% differentiation.

What is claimed is:

1. Method for treating a host having NIDDM, comprising the step of administering to said host a composition
5 comprising a pharmaceutically effective amount of an RXR agonist.
2. The method of claim 1, further comprising the step of administering to said host a composition comprising a
10 pharmaceutically effective amount of a PPAR γ agonist.
3. The method of claim 1, wherein said composition further comprises a pharmaceutically effective amount of a
PPAR γ agonist.
15
4. The method of claim 1, wherein said RXR agonist is an RXR specific agonist.
5. The method of claim 4, wherein said RXR agonist is
20 LG 100268.
6. The method of claim 4, wherein said RXR agonist is LGD 1069.
- 25 7. The method of claim 1, wherein said RXR agonist also activates a retinoic acid receptor.
8. The method of claim 7, wherein said RXR agonist is 9-cis retinoic acid.

9. The method of claim 2, wherein said PPAR γ agonist is a thiazolidinedione compound.

10. The method of claim 9, wherein said
5 thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075 and darglitazone.

10 11. The method of claim 1, further comprising the step of administering to said host a composition comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

15 12. The method of claim 3, wherein said composition further contains a pharmaceutically effective amount of insulin, insulin mimetic, or insulin sensitizer.

20 13. A pharmaceutical composition adapted for the treatment of NIDDM comprising

(a) a pharmaceutically effective amount of an RXR agonist; and

(b) a pharmaceutically acceptable carrier.

25 14. The composition of claim 13, further comprising a pharmaceutically effective amount of a PPAR γ agonist.

15. The composition of claim 13, wherein said RXR agonist is LG 100268.

5 16. The composition of claim 13, wherein said RXR agonist is LGD 1069.

17. The composition of claim 13, wherein said RXR agonist is 9-cis retinoic acid.

10 18. The composition of claim 14, wherein said PPAR γ agonist is a thiazolidinedione compound.

15 19. The composition of claim 18, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075 and darglitazone.

20 20. The composition of claim 14 further comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

25 21. Method for increasing glucose uptake in adipose or muscle tissue, comprising the step of administering to said tissue a composition comprising a pharmaceutically effective amount of an RXR agonist.

22. The method of claim 21, further comprising the step of administering to said tissue a composition comprising a pharmaceutically effective amount of a PPAR γ agonist.

5 23. The method of claim 21, wherein said composition further comprises a pharmaceutically effective amount of a PPAR γ agonist.

10 24. The method of claim 21, wherein said RXR agonist is an RXR specific agonist.

25. The method of claim 24, wherein said RXR agonist is LG 100268.

15 26. The method of claim 24, wherein said RXR agonist is LGD 1069.

27. The method of claim 21, wherein said RXR agonist also activates a retinoic acid receptor.

20

28. The method of claim 27, wherein said RXR agonist is 9-cis retinoic acid.

29. The method of claim 22, wherein said PPAR γ agonist is a thiazolidinedione compound.

25

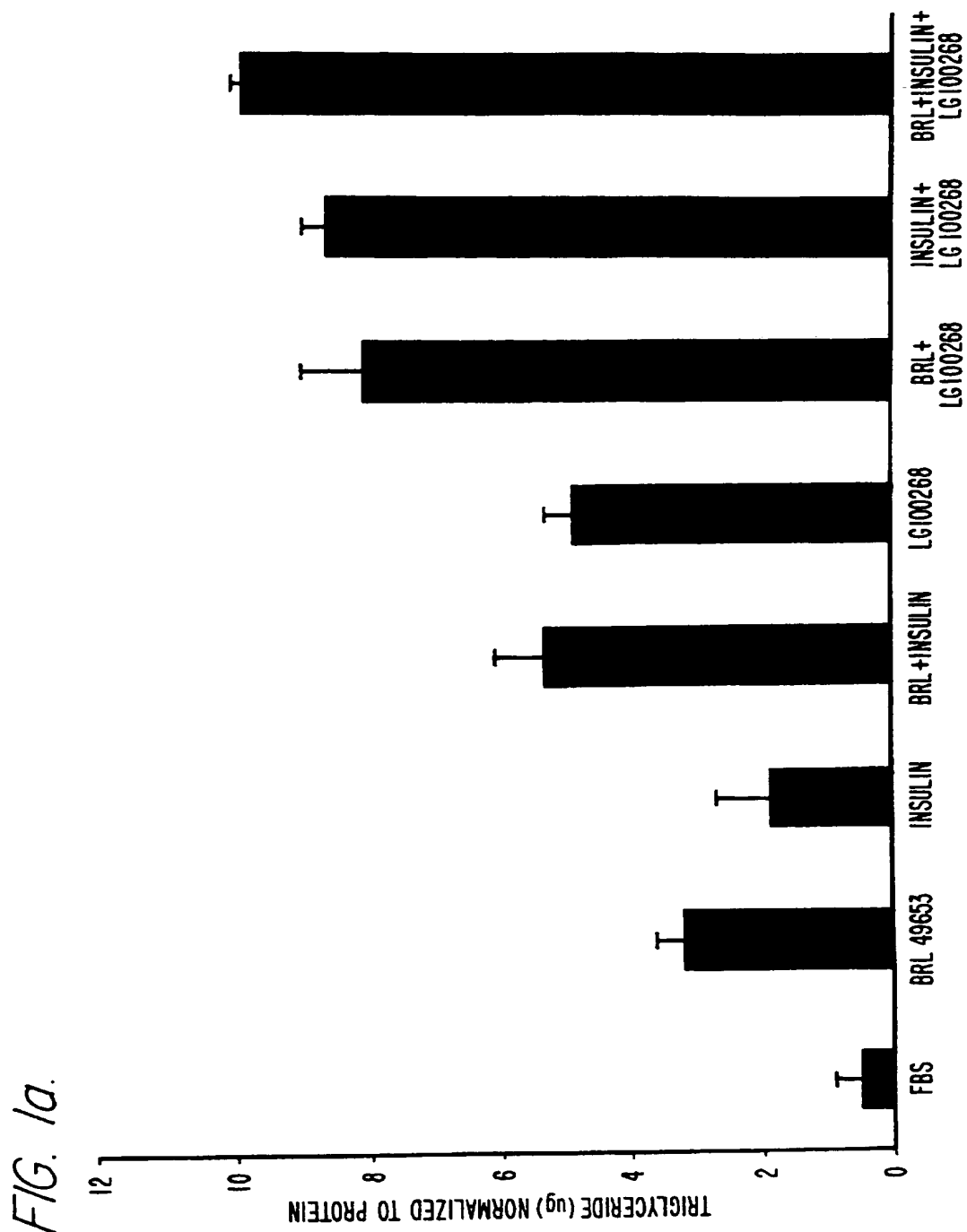
30. The method of claim 29, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone,

ciglitazone, WAY-120,744, englitazone, AD 5075 and darglitazone.

5 31. The method of claim 21, further comprising the step of administering to said tissue a composition comprising a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

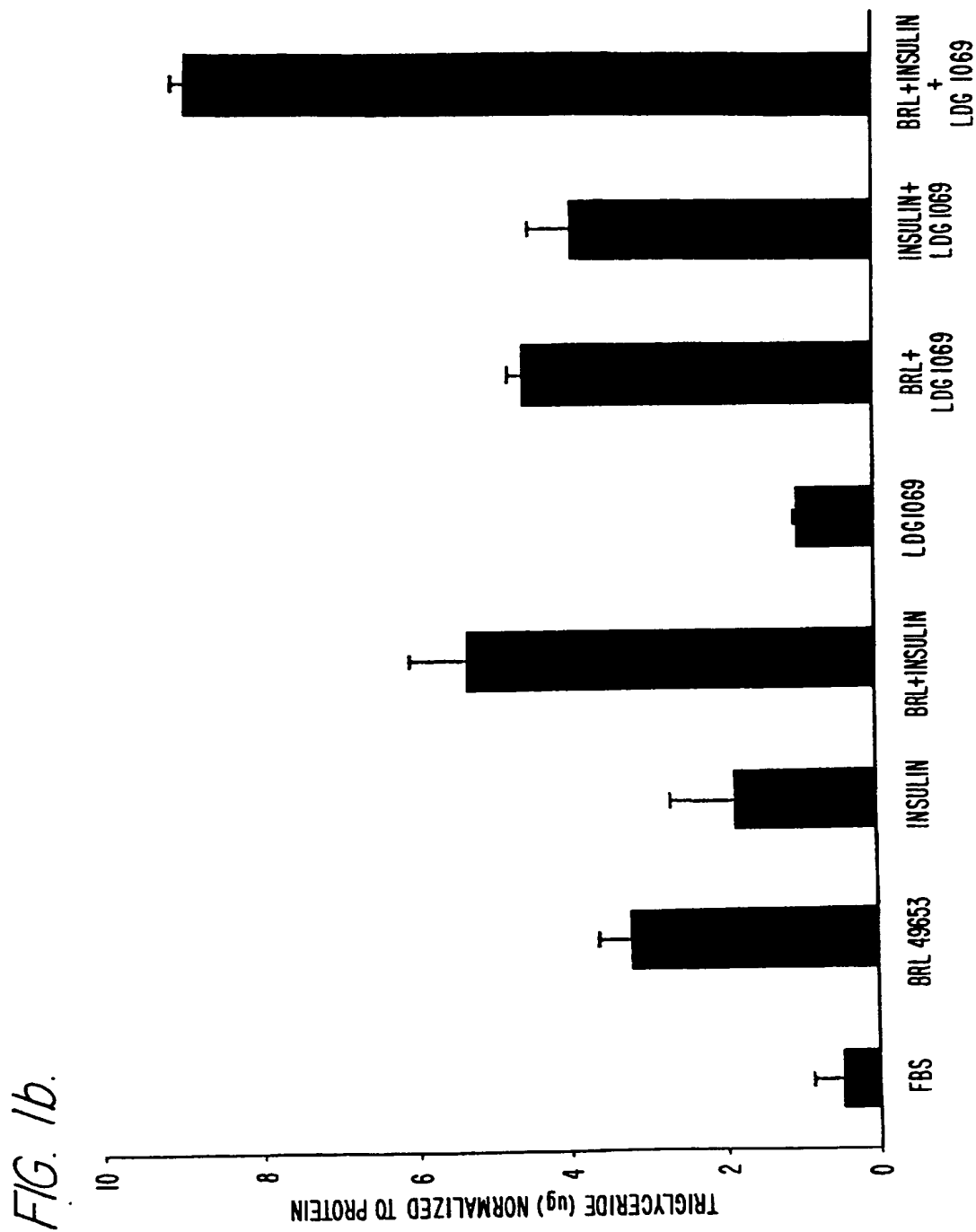
10 32. The method of claim 23, wherein said composition further contains a pharmaceutically effective amount of insulin, insulin mimetic, or insulin sensitizer.

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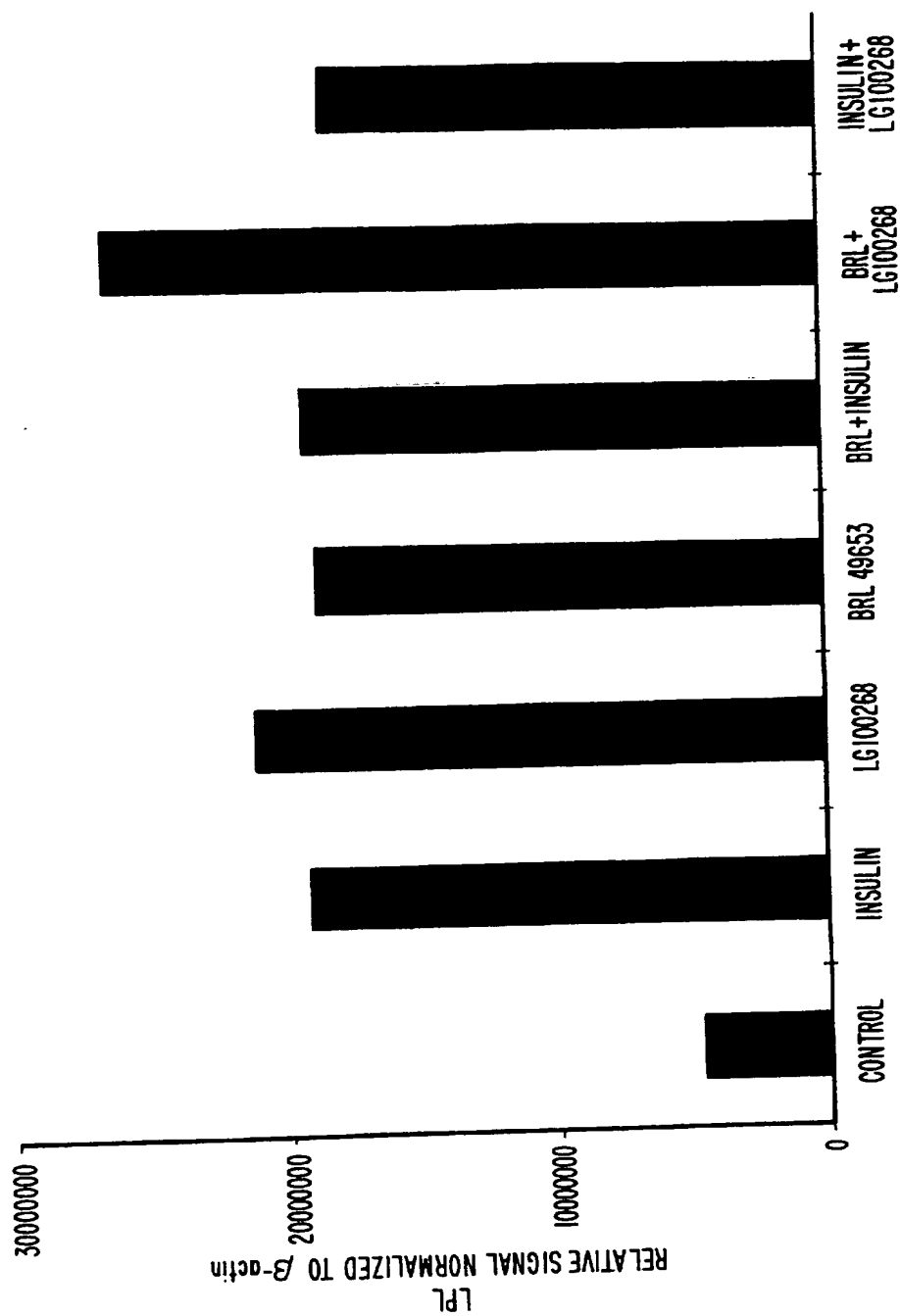
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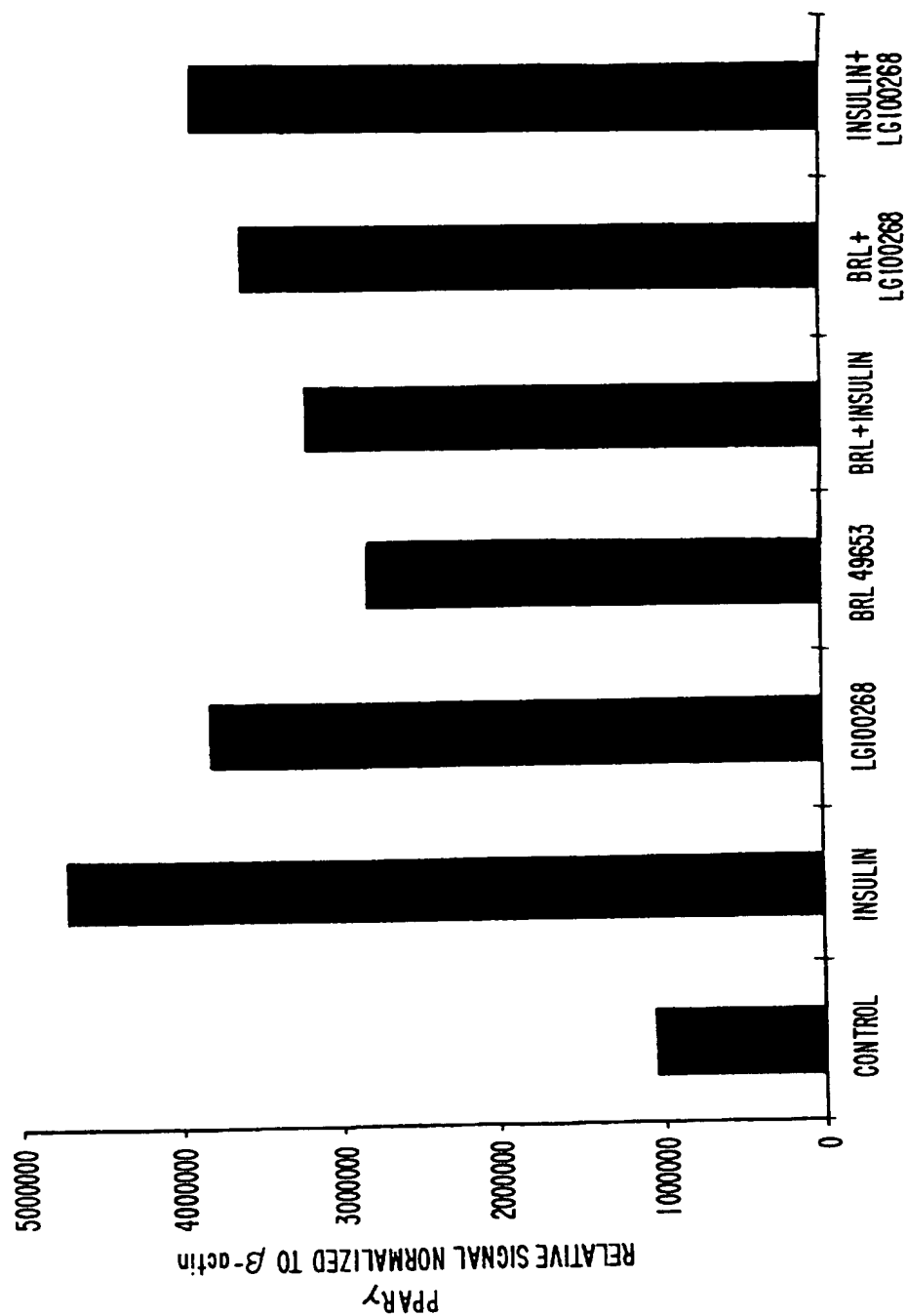
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FIG. 2a.



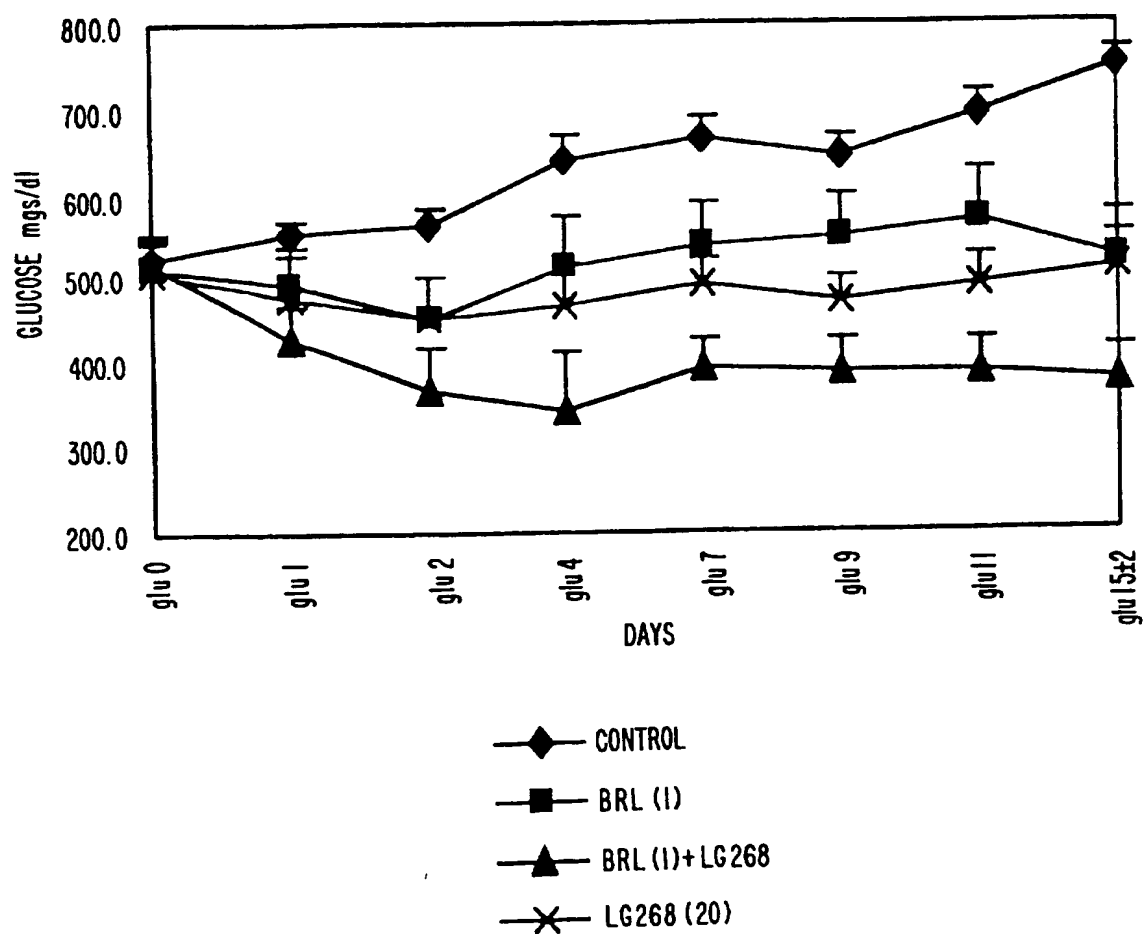
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FIG. 2b.



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FIG. 3.



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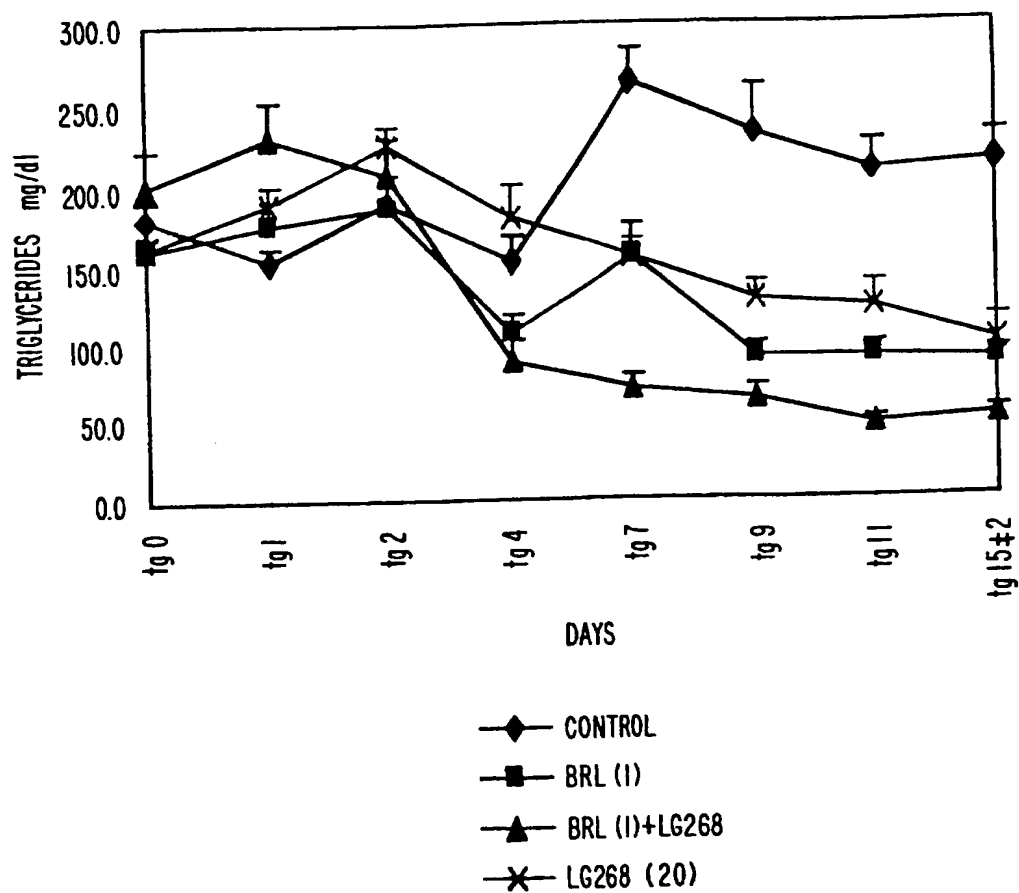
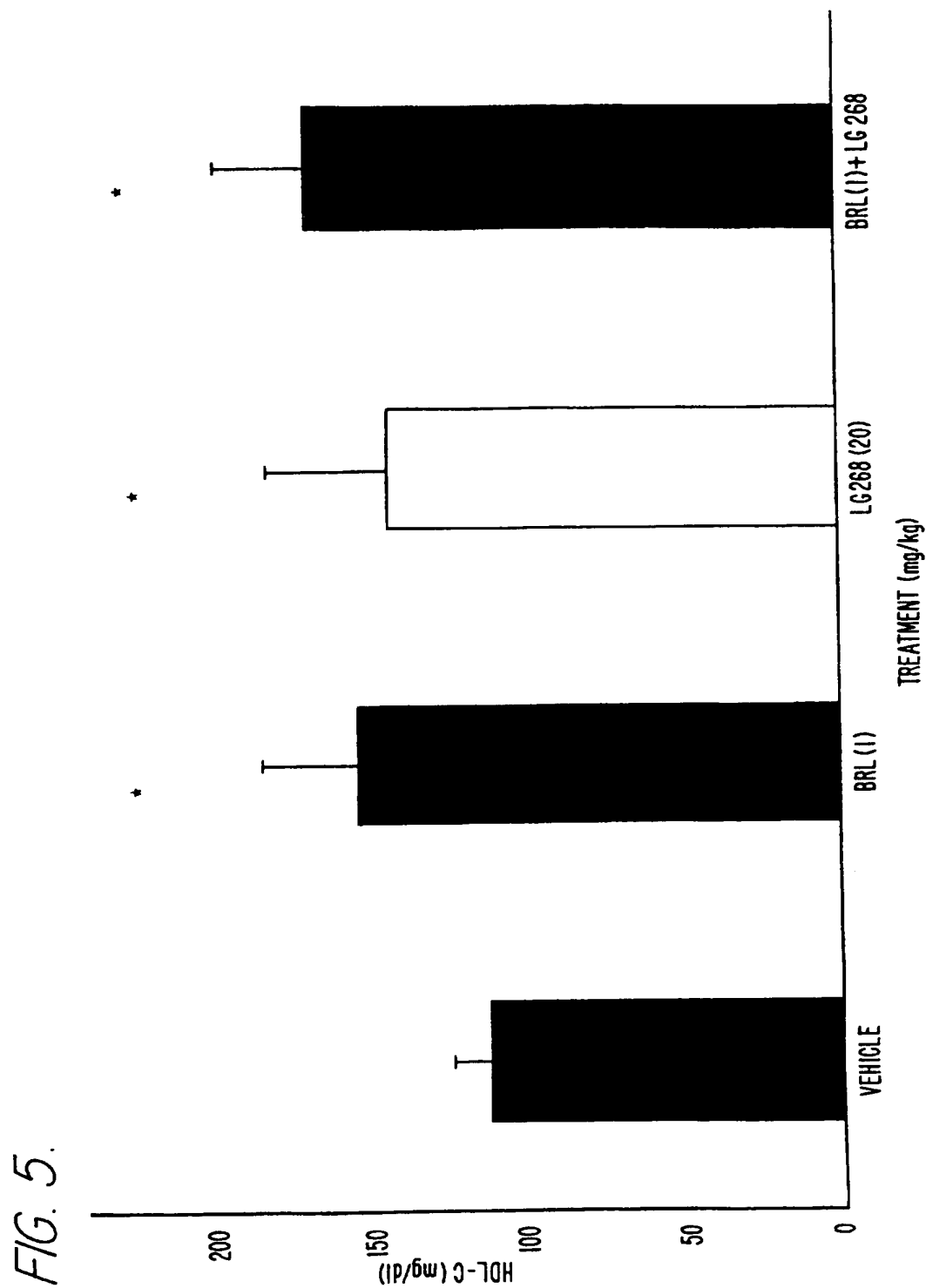


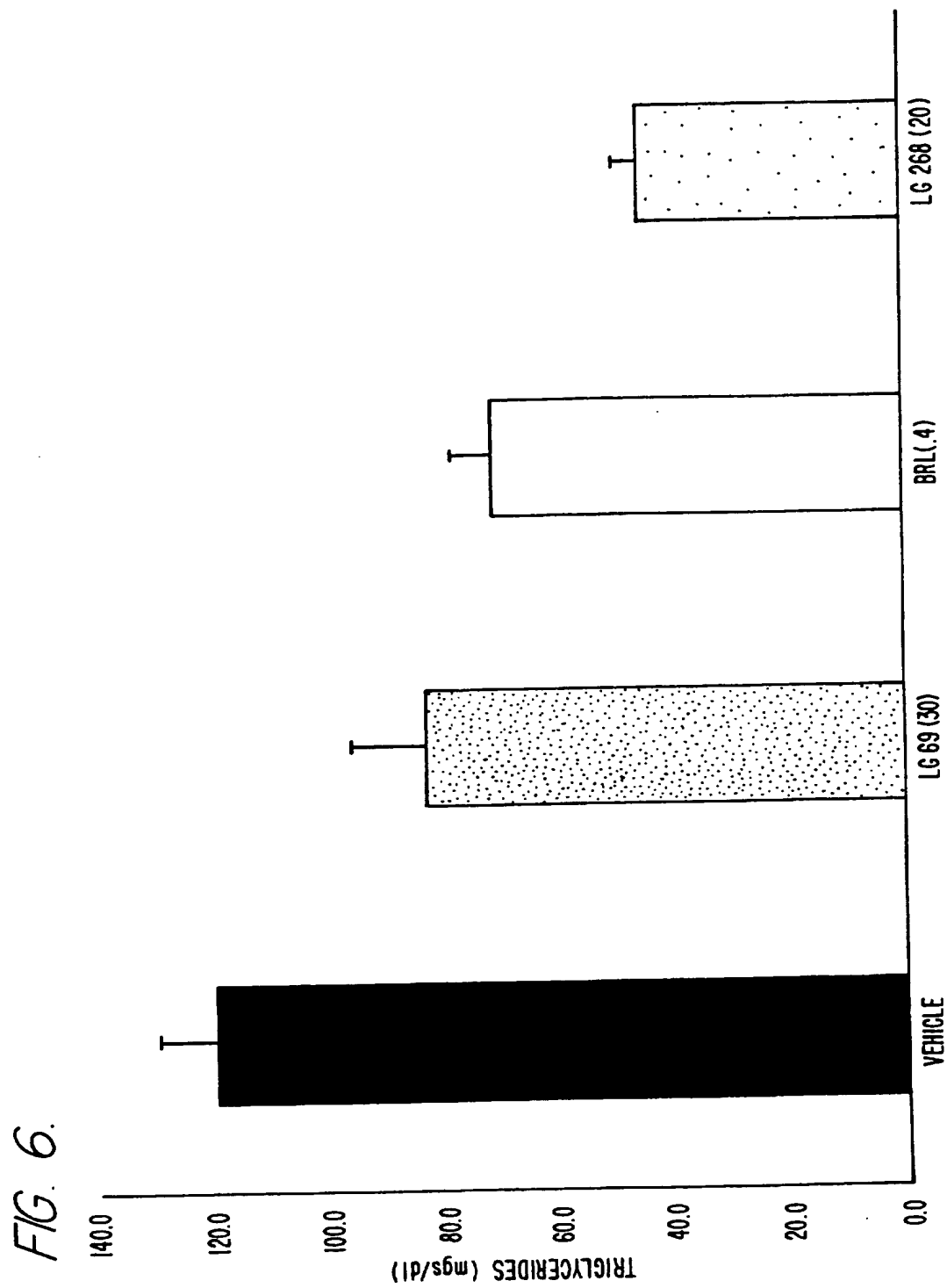
FIG. 4.

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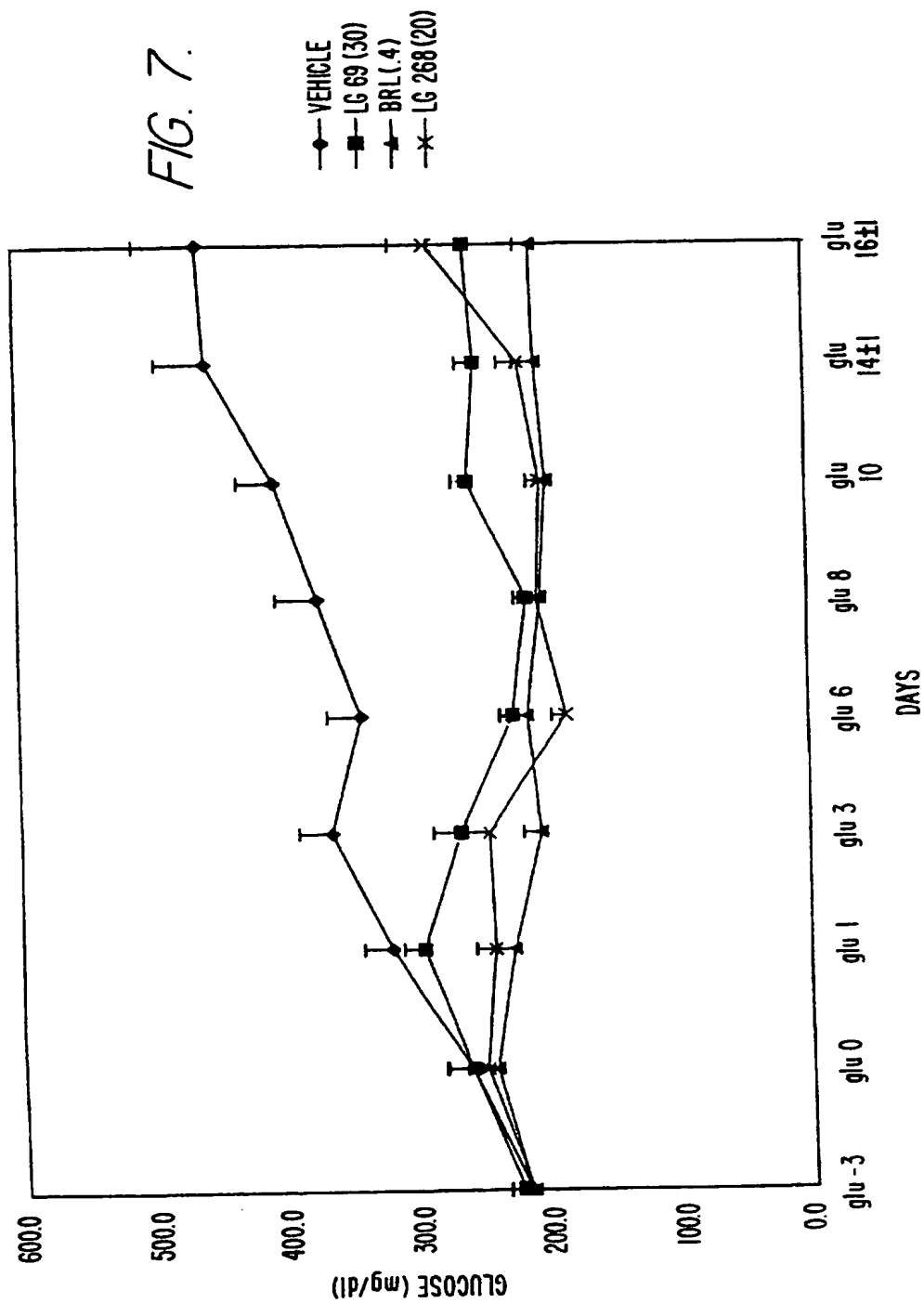
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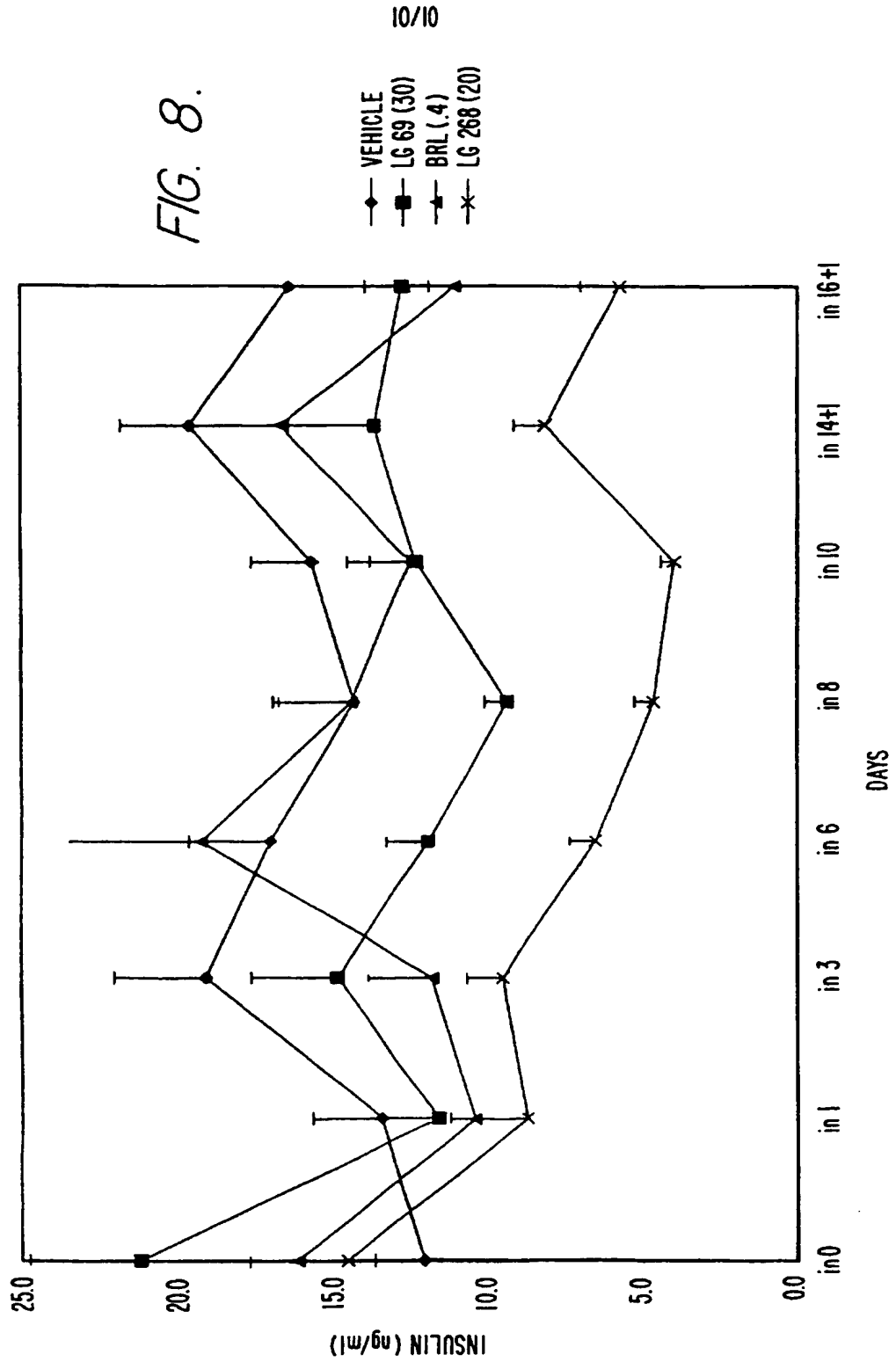
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FIG. 7.



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FIG. 8.



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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/14904

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/19 A61K31/455 A61K31/20 //(A61K31/455,31:44,
31:425,31:20,31:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DIABETOLOGIA, vol. 39, 5 September 1996, page A234 XP000613679 J.M. LENHARD ET AL.: "ANALYSIS OF THIAZOLIDINEDIONE, BIGUANIDE AND RETINOID EFFECTS ON ADIPOGENESIS AND THE NUCLEAR RECEPTORS PPARGamma AND RXR" see abstract	1-4,9, 10,13, 14,18,19
P,X	EP 0 698 392 A (L'OREAL) 28 February 1996 see the whole document --- -/--	1-3,7,8, 13,14,17

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search

16 January 1997

Date of mailing of the international search report

31.01.97

Name and mailing address of the ISA

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Authorized officer

Hoff, P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/14904

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 21146 A (LIGAND PHARMACEUTICALS) 28 October 1993 cited in the application see abstract see page 10, line 34 - page 17 see page 66, line 13 - page 67, line 5; claims; examples 44-51; table 5 ---	13-17
X	EP 0 552 624 A (HOFFMANN-LA ROCHE) 28 July 1993 see the whole document ---	13,17
X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 38, no. 16, 4 August 1995, pages 3146-3155, XP000615461 M.F. BOEHM: "DESIGN AND SYNTHESIS OF POTENT RETINOID X RECEPTOR SELECTIVE LIGANDS THAT INDUCE APOPTOSIS IN LEUKEMIA CELLS" see the whole document ---	13,15-17
A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 204, no. 2, 1994, pages 498-504, XP000615920 I. SAFONAVA ET AL.: "FATTY ACIDS AND RETINOIDS ACT SYNERGISTICALLY ON ADIPOSE CELL DIFFERENTIATION" see the whole document ---	1-32
A	GENES & DEVELOPMENT, vol. 8, no. 10, 1994, pages 1224-1234, XP000577793 P. TONTONOV ET AL.: "mPPARGamma2: TISSUE-SPECIFIC REGULATOR OF AN ADIPOCYTE ENHANCER" cited in the application see the whole document ---	1-32
A	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 22, September 1995, pages 12953-12956, XP000577082 J.M. LEHMANN ET AL.: "AN ANTIDIABETIC THIAZOLIDINEDIONE IS A HIGH AFFINITY LIGAND FOR PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA" cited in the application see the whole document -----	1-32

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/14904

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-12, 21-32
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:

Please see next page

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

A compound cannot be sufficiently characterised by its pharmacological profile or its mode of action as it is done by expressions like "RXR agonist" or "PPARY agonist". In view of the large number of compounds which are theoretically contained within those definitions, the search had to be restricted on economic grounds to the compounds specifically mentioned in claims 5,6,8 and 10. (PCT Art.6; Guidelines Part B, chapter II. 7 last sentence and chapter III, 3.7)

Claims searched incompletely: 1-4, 7, 11-14, 20-24, 27, 31-32

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PLT/US 96/14904

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-698392	28-02-96	FR-A- 2723315 AU-A- 2723295 JP-A- 8059459	09-02-96 29-02-96 05-03-96
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WO-A-9321146	28-10-93	AU-A- 4118893 AU-A- 5586894 AU-A- 5664294 AU-A- 6225894 BR-A- 9307784 CA-A- 2133587 CA-A- 2153235 CA-A- 2153236 EP-A- 0637297 EP-A- 0678086 EP-A- 0678087 JP-T- 8511027 JP-T- 8505852 JP-T- 8505359 NO-A- 943943 WO-A- 9415901 WO-A- 9504036 WO-A- 9415902	18-11-93 15-08-94 28-02-95 15-08-94 14-11-95 28-10-93 21-07-94 09-02-95 08-02-95 25-10-95 25-10-95 19-11-96 25-06-96 11-06-96 21-12-94 21-07-94 09-02-95 21-07-94
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EP-A-552624	28-07-93	AU-B- 667864 AU-A- 3186193 CA-A- 2087251 CN-A- 1074826 HU-A- 9500256 JP-A- 6107542 NZ-A- 245686 US-A- 5428071 ZA-A- 9300286	18-04-96 29-07-93 23-07-93 04-08-93 28-09-95 19-04-94 26-05-95 27-06-95 22-07-93
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